

# DEVELOPMENT OF THE EGGS AND EARLY LARVAE OF THE AUSTRALIAN SMELT, *RETROPINNA SEMONI* (WEBER)

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(Plates vii-ix)

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## *Synopsis*

*Retropinna semoni* (Weber) is a small freshwater fish, occurring widely in eastern Australia. Its eggs, embryonic development, and early larvae are described.

## INTRODUCTION

The Australian smelt, *Retropinna semoni* (Weber), has a wide distribution in eastern Australia, occurring throughout the great Murray-Darling River system and also in coastal streams (Munro, 1957). The species grows to a length of only 10 cm. and is unimportant either commercially or to the angler. However, in many areas it is extremely abundant and is preyed upon by several of the larger fishes utilized by man (Butcher, 1945; personal records).

The following account of the eggs, embryonic development, and early larvae of *R. semoni* is based on studies carried out at the Inland Fisheries Research Station, Narrandera, New South Wales. The studies were initiated following evidence of natural breeding in one of the experimental ponds at this Station.

## METHODS

Following the discovery of larval stages of *R. semoni* in an experimental pond on September 22, 1961, collections of adults in reproductive condition were made from the pond later the same day and also on September 23.

A first attempt to obtain fertilized eggs was made by adding milt to ova, apparently ripe but not strippable, removed from a female by dissection. No fertilization was achieved.

A second attempt proved successful. On this occasion the ova were readily stripped from a female, measuring 75 mm. total length, by applying slight pressure on the abdomen. Milt was expressed from a male, measuring 73 mm., in a similar manner, and placed onto the eggs held in a Petri dish. Water was immediately added to cover the eggs and the whole mixed by gentle shaking. Approximately 20 minutes later the eggs were washed with several changes of water and then transferred to a shallow enamel tray. This was immersed in a deeper tray, through which water was circulated. On the eighth day, when hatching appeared imminent, the water circulation was stopped to avoid loss of larvae in the overflow.

The temperature of the water flowing over the eggs was not controlled, but for the greater part of the hatching period fluctuated within the range 15.5 to 18.0°C. On two occasions, for periods of three to four hours, the temperature fell to about 13°C.

Measurements were made with an ocular micrometer. The photographs were taken using transmitted light.

Efforts were made to rear the larvae in glass and plastic containers in the laboratory. However, although various kinds of finely ground food were supplied, no feeding was observed and the longest that any larvae survived was 8 days.

### THE EGGS

The eggs when stripped are spherical, with an average diameter of 0.80 mm. Following fertilization they swell to an average diameter of 0.95 mm. They sink in fresh water and are strongly adhesive, becoming firmly stuck to the bottom of the hatching tray. The yolk is pale amber in colour. Initially it completely fills the egg, but quite a large perivitelline space is formed during the swelling of the thin capsule. One to several large and many small oil globules are present within the yolk.

### EMBRYONIC DEVELOPMENT

The formation of the blastodisc commences almost immediately upon fertilization. Cytoplasm, which has hitherto invested the yolk in an invisible layer, slowly accumulates at the animal pole of the egg (Plate vii, fig. 1). When concentration of the cytoplasm is complete the formed blastodisc is of a lenticular shape, the surface of the yolk immediately opposite having flattened (Plate vii, fig. 2).

The first three or four cleavages occur within 3 hours following fertilization. These early cleavages, at least up to and including the fourth, take place regularly throughout the blastoderm, the blastomeres formed being fairly uniform in size (Plate vii, fig. 3). In a small number of eggs the cleavage rate was slower and in a few the cleavages were irregular, so that unequal blastomeres were formed. However, these eggs having retarded and/or obviously irregular development all died during the first two days. Subsequent mortalities until after hatching were few and development progressed more or less uniformly in all eggs.

Sixteen hours after fertilization the blastodermal cap has been formed. It is of a similar lenticular shape to that of the blastodisc but more opaque (Plate vii, fig. 4).

About 22 hours after fertilization the germ ring has reached approximately an equatorial position about the yolk. It is slightly thickened and appears to constrict the yolk sphere as it advances over it (Plate vii, fig. 5). The thickening of the germ ring is more pronounced on one side than the other, the thickened portion marking the posterior pole at which the embryonic shield develops.

The embryo is clearly evident and shows marked development at 41 hours. It extends approximately two-thirds the way around the yolk, and is noticeably thickened in the cephalic region. The thin blastodermal layer now almost fully encloses the yolk, except at the blastopore, situated just posteriorly to the tail end of the embryo, through which there is a slight bulging of the yolk.

About 47 hours after fertilization, the optic vesicles are easily discernible (Plate vii, fig. 6). The embryo is much thickened along its whole length, especially in the cephalic region, and dorsally protrudes markedly into the perivitelline space. Kupffer's vesicle, a small transparent sphere lying ventrally near the posterior end of the embryo, has appeared.

Considerable differentiation has occurred in the embryo by 66 hours after fertilization (Plate viii, figs 7, 8). The eyes are now very clear and the pupils have developed. The head is further enlarged and the lobes of the brain are apparent. Auditory capsules are present and more than 30 mesodermal somites are distinguishable. The embryo almost fully encircles the yolk, which is slightly constricted around the line of contact. To this point no pigmentation has been developed.

The embryo more than fully encircles the yolk at 95 hours, the tail slightly overlapping the head (Plate viii, fig. 9). The heart, which was not distinguished at 66 hours, is now easily seen. It is situated just under and posterior to the



eyes, and pulsates quite regularly. The first pigmentation has now appeared as series of melanophores on the sides of the body, along portion of the midline and to a lesser extent along the ventral edge. Otoliths have developed within the auditory capsules. The first movements, slight twitchings, of the embryo were observed at this stage.

At about 113 hours the embryo extends approximately one and a quarter times around the yolk, and the eyes are becoming quite heavily pigmented. Movements of the embryo are now more frequent, the posterior portion of the body being detached from the yolk and moving freely.

From this stage until hatching there is a continued increase in the length of the embryo, so that at 137 hours it encircles the yolk approximately one and a half times (Plate viii, fig. 10) and at 165 hours about two times (Plate viii, figs 11 and 12). There is a marked lateral expansion of the head, causing it to be roughly triangular in shape by 165 hours (Plate viii, fig. 12). The melanophores along the body increase in number, becoming more uniform and pronounced, and others develop at about 130 hours over the yolk sac. The dorsal and ventral fin-folds are clearly evident at about 130 hours.

Towards hatching the embryo moves almost incessantly, the tail twisting and switching from side to side and at intervals the whole embryo revolves completely within the egg capsule. The pectoral fins, now evident as transparent fan-like structures slightly posterior to the auditory capsules, become active and beat rapidly for increasing periods of time from about 210 hours onwards.

Hatching commenced at 216 hours after fertilization and all the larvae had emerged from the egg capsules by 225 hours.

#### THE LARVAE

The newly hatched larvae (Plate ix, figs 13 and 14) are extremely elongate in form, the average total length being 4.61 mm. The head is inflected downwards and is anteriorly rounded, so that the eyes appear ventrally placed. The mouth is present as a small opening situated below the eyes, but is probably non-functional for the first day or so after hatching. The auditory capsules are comparatively large and protrude prominently from the sides of the head. A small mass of yolk contained in an elongate, ovoid sac is still present at hatching. A single oil globule is present within the anterior end of the yolk sac. The hind portion of the alimentary canal is clearly evident as a long, straight tube and the anal opening is situated two-thirds the way along the body. Both the dorsal and ventral fin-folds are fairly uniform in height throughout and are continuous with the caudal fin-fold, which is slightly more expanded and lobate.

On hatching, the larvae congregated near the surface and sides of the hatching tray. For most of the time they remained fairly passive, normally orientated horizontally with dorsal side uppermost, but occasionally, particularly if disturbed, swimming actively with apparently well directed movements.

One day after hatching, the yolk sac and contained oil globule are both much reduced in size (Plate ix, fig. 15). The head has now pivoted forwards and upwards, so that the mouth is situated more anteriorly and the eyes lie slightly more dorsally than in the newly hatched larva. The straightening of the head contributes to a relatively large increase in length, so that the average total length attains 5.25 mm.

After two days the yolk has been almost completely absorbed and the oil globule has disappeared (Plate ix, fig. 16). Up to this time there is evidence of continued differentiation in the larvae, particularly in the head region. The jaws are now well developed, the auditory capsules further enlarged, and the pectoral fins larger, stronger and much easier to see. Compared with the increase in length over the first day, that during the second is small, the average total length of the two-day larvae being 5.29 mm.

Following the complete utilization of the yolk on the third day, development of the larvae almost ceased and there was evidence of emaciation. This was undoubtedly due to unsuitability of food provided. A slight increase in length occurred to the fifth day, the average total length of larvae then surviving being 5.51 mm. No further growth was recorded and the single eight-day larva measured only 5.30 mm., the shrinkage possibly being due to a natural consolidating of tissues, but more probably to the larva having to resort to its own body substance for nourishment.

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### EXPLANATION OF PLATES VII-IX

#### Plate vii

Fig. 1. Egg 17 min. after fertilization. Incomplete blastodisc.—Fig. 2. Egg 1 hr. after fertilization. Complete blastodisc.—Fig. 3. Egg 3 hr. after fertilization. Sixteen cells.—Fig. 4. Egg 16 hr. after fertilization. Blastodermal cap.—Fig. 5. Egg 22 hr. after fertilization. Germ ring at equatorial position.—Fig. 6. Egg 47 hr. after fertilization. Blastopore closed, embryo protruding into perivitelline space, and optic vesicles evident.

#### Plate viii

Figs 7, 8. Egg 66 hr. after fertilization. Brain lobes, pupils of eyes, auditory capsules, and somites distinct.—Fig. 9. Egg 95 hr. after fertilization. Embryo completely encircling yolk, first pigmentation on body.—Fig. 10. Egg 137 hr. after fertilization. Embryo encircling yolk approx  $1\frac{1}{2}$  times, eyes heavily pigmented, melanophores over yolk sac.—Figs 11, 12. Egg 165 hr. after fertilization. Very advanced embryo, encircling yolk approx. 2 times.

#### Plate ix

Figs 13, 14. Newly hatched larva. Average length 4.61 mm.—Fig. 15. One day old larva. Average length 5.25 mm.—Fig. 16. Two days old larva. Average length 5.29 mm.